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PREDATORY BEHAVIOR OF *REPIPTA FLAVICANS* STÅL (HEMIPTERA: REDUVIIDAE), A NATURAL ENEMY OF DIABROTICINA (COLEOPTERA: CHRYSOMELIDAE)

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Abstract.—Predation bioassays were conducted to evaluate the predatory behavior of *Repipta flavicans* Stål (Hemiptera) under laboratory conditions. In the field, this predator was identified as a natural enemy of the Diabroticina beetle, *Acalymma blomorum* Munroe and Smith (Coleoptera: Chrysomelidae). Predator specificity, functional response, and the potential tritrophic effect of the secondary compounds (cucurbitacins) sequestered by prey were determined. An effect of the elytra coloration of the prey was not observed, but in some cases prey body size seemed to have had an effect on predation. The results of the tritrophic level assay suggested that cucurbitacins sequestered by *A. blomorum* did not have a repellent or toxic effect on this predator. By contrast, plant secondary compounds present in prey increased the longevity of *R. flavicans* females. Cucurbitacins were present in the predators after ingesting *A. blomorum*.

Resumen.—Se realizaron bioensayos de depredación para evaluar el comportamiento predatorio de *Repipta flavicans* Stål (Hemiptera: Reduviidae) bajo condiciones de laboratorio. En campo, este depredador fue identificado como enemigo natural de la Diabroticina *Acalymma blomorum* Munroe and Smith (Coleoptera: Chrysomelidae). Además, se determinó su especificidad, respuesta funcional y el efecto tritrófico de los compuestos secundarios (cucurbitacinas) obtenidos por las presas. No se observó que la coloración de los élitros, de los escarabajos utilizados, tuviera un efecto sobre el depredador, sin embargo, en algunos casos el tamaño corporal parece haber afectado la elección del mismo. Los resultados sugieren que las cucurbitacinas retenidas por *A. blomorum* no tuvieron un efecto repelente o tóxico en el depredador. Por el contrario, la presencia de los compuestos secundarios en las presas incrementaron la longevidad de las hembras *R. flavicans* y estuvieron presentes en los depredadores después de que ingirieron presas *A. blomorum*.

Key Words: *Acalymma blomorum*, tritrophic effect, cucurbitacins, Diabroticina, predation, *Repipta flavicans*

Harpocaptorine species (Hemiptera: Reduviidae) are common predators on other insects throughout the Neotropical Region (Schuh and Slater 1995). *Repipta flavicans* Stål is distributed in the central states of Mexico. In a previous paper, it was reported for the first time as a predator of *Acalymma blomorum* Munroe and Smith in its natural habitat (Gámez-Virués et al. 2003). This prey species belongs to the Diabroticina beetles (Chrysomelidae) which are native to Mexico and Central America (Webster

1895). Their main economically important hosts are maize, beans, and cucurbits. For that reason, several species of *Acalymma* are considered important pests (Burkness and Hutchison 1998), and numerous studies have been conducted on these pests (Brust and Foster 1995, Kuhlman and Van der Burgt 1998, Lance 1988).

Nevertheless, little information exists about the natural enemies of Diabroticina in their native habitat. The reduviid *Castolus tricolor* Champion was recognized as a predator of Diabroticina in Costa Rica (Risch 1981). Eben and Barbercheck (1996) reported the spider *Oxyopes salticus* Hentz (Oxyopidae) and three species of Reduviidae as predators of *A. blomorum* and *Diabrotica balteata* LeConte in Veracruz, Mexico. Studies on the use of biocontrol agents against Diabroticina species were performed with entomopathogenic nematodes (Jackson 1996), entomopathogens fungi (Tallamy et al. 1998), and recently with flies of the family Tachinidae (Zhang et al. 2004). Furthermore, Howe et al. (1976) reported for the first time that ingestion of cucurbitacins by Diabroticina can be related to protection against birds and other insectivorous vertebrates. The hypothesis of chemical defense has since been studied with a diverse array of natural enemies, however, no clear pattern has been detected (Ferguson and Metcalf 1985, Nishida et al. 1992, Brust and Barbercheck 1992, Barbercheck et al. 1995).

The objective of this study was to characterize the predatory behavior and specificity of *R. flavicans* on Diabroticina beetles. The effect of cucurbitacins present in the diet of the beetle prey was also evaluated for this predatory bug.

Materials and Methods

Predators.—*Repipta flavicans* adults were collected in the field where they were observed preying on *A. blomorum*. They were found in particularly high numbers on two cucurbit species: *Cucurbita okeechobeensis* ssp. *martinezii* L. Bailey, a wild bitter cucurbit, and *C. moschata* (Lam.) Poiret, an edible cucurbit. Predatory bugs were kept under laboratory conditions (25 ± 3°C; 13:11, L:D). They were provided daily with adults of *D. balteata*, *D. porracea* Harold and larvae of *Anastrepha ludens* (Loew) (Diptera: Tephritidae) as food. A laboratory colony was initiated with the eggs deposited by the field-captured bugs, using methods described by Gámez-Virués et al. (2003). Nymphs used later in bioassays were kept away from Diabroticina species and were fed only with *A. ludens* larvae.

Diabroticina prey.—For all bioassays, adults Diabroticina beetles were used as prey. Beetles were collected on the same cucurbits than predators. Some beetles were fed with fruits of *Cucurbita pepo* L., a cultivar free of cucurbitacins, and artificial diet (Branson et al. 1975) during 28 d. This allowed excretion of previously sequestered bitter compounds (treatment 1 = without cucurbitacins, SC). Other beetles were fed with bitter fruits of *C. o. martinezii*, that are rich in cucurbitacins, for a period of 7 to 10 d (Andersen et al. 1988) (treatment 2 = with cucurbitacins, CC). In addition, five other Diabroticina species collected in the field on the same hosts were used in bioassays: *A. innubum* (Fabricius), *A. trivittatum* Mannerheim, *Cerotoma atrofasciata* Jacoby, *D. balteata*, and *D. tibialis* Baly. Beetles were kept on the SC diet for at least 28 d before they were used in bioassays. The six Diabroticina species were separated by species and kept in cages of 30 cm × 30 cm × 30 cm, under laboratory conditions.

Functional response of *R. flavicans*.—Predation of *R. flavicans* was evaluated according to prey density. Naive females were used as predators. Each female was offered 1, 2, 5, 10, 20, or 30 individuals of *A. blomorum* as prey. Predation was evaluated after 1 h, 8 h, and 24 h. Transparent plastic containers (5 l) with a twigs to simulate a natural environment were used in the assay. Twelve repetitions were made for each prey density. Results were analyzed by one-way

ANOVA ($P < 0.05$) with SigmaStat™ statistical software version 2.0 (Jandel Scientific 1992–1997).

Predation bioassays.—Twenty-four hours before each bioassay, bugs were placed individually in 250 ml cages and were deprived of food. Bioassays concluded when bugs attacked a beetle or after 48 h. As a control, five *A. blomorum* fed on SC diet and five *A. blomorum* fed on CC diet were placed in plastic containers (250 ml) and their mortality without the presence of a predator was recorded after 48 h.

1) Specificity. This was evaluated by observing whether the predatory behavior of *R. flavicans* was affected by coloration of the elytra or the body size of its prey. Six Diabroticina species, that coexist as adults on the same *Cucurbita* spp., were used.

Coloration: Two Diabroticina species were offered to each predator in three combinations: one *A. blomorum* and another species with a) similar coloration: *A. trivittatum,* or b) different coloration: *C. atrofasciata* or *D. balteata.*

Body size: Two beetles were offered to each predator in choice tests: one *A. innubum,* with similar coloration that *A. blomorum,* and one *D. tibialis,* both species of greater size than *A. blomorum.* For these assays, ten female and ten male *R. flavicans* from the laboratory colonies were used. The results were analyzed with a chi-square test ($P<0.05$, SigmaStat).

2) Effect of the diet of *A. blomorum* on predation by *R. flavicans.* Two *A. blomorum,* one of each diet treatment, were offered to each predator in a choice situation. The bioassay was finished when the predator attacked one of the beetles or after a period of 48 h. Twenty females collected in the field, and 10 females and 10 males obtained in laboratory were used. The results were analyzed with a t-test for independent samples ($P < 0.05$, SigmaStat).

3) Effect of the diet of *A. blomorum* on the longevity of *R. flavicans.* Each predator was offered two *A. blomorum* fed on SC diet or two *A. blomorum* fed on CC diet every 48 h, during their entire adult lifetime. Twenty-four hours after providing the prey, the number of predated beetles and the survival of each bug were recorded. Only *R. flavicans* females raised under laboratory conditions were used. Nine repetitions per treatment were made. The results were analyzed with a t-test for independent samples ($P<0.05$, SigmaStat).

Qualitative detection of cucurbitacins in *A. blomorum* and *R. flavicans.*—A variation of the method established by Halaweish and Tallamy was used (1993). Cucurbitacins were detected with thin layer chromatography (TLC). The samples were obtained by grinding 15 *A. blomorum* beetles of each treatment in 3 ml ethanol (70%). Each extract was filtered and the solid phase was discharged. To the extract, 2 ml hexane were applied, it was shaken slowly in a separatory funnel, and the hexane phase was discharged (2×). Afterwards, 2 ml chloroform were applied, and the ethanolic phase was discharged. Finally, the extract was evaporated until the solvent was eliminated and a yellowish residue was obtained. In order to determine if the bugs acquired the plant compounds sequestered by their prey, both females that were fed during their adult life exclusively with *A. blomorum* fed on SC diet and females fed with *A. blomorum* fed on CC diet were used (bioassay 3). All dead *Repipta* females per treatment were stored individually in ethanol (70%). Extracts were obtained with the same method as above described for *A. blomorum.* The samples of *A. blomorum* and *R. flavicans* were applied on silica gel 60 TLC plates (without fluorescent indicator, MERCK). Plates were developed with ethyl acetate: toluene (6:4). Plates were observed under UV light to 254 nm. The retention factor (Rf) of each visible compound was determined and compared with standard values for cucurbitacin B, D, and I.

Results

Functional response of *R. flavicans.*—Predation was proportional to prey density

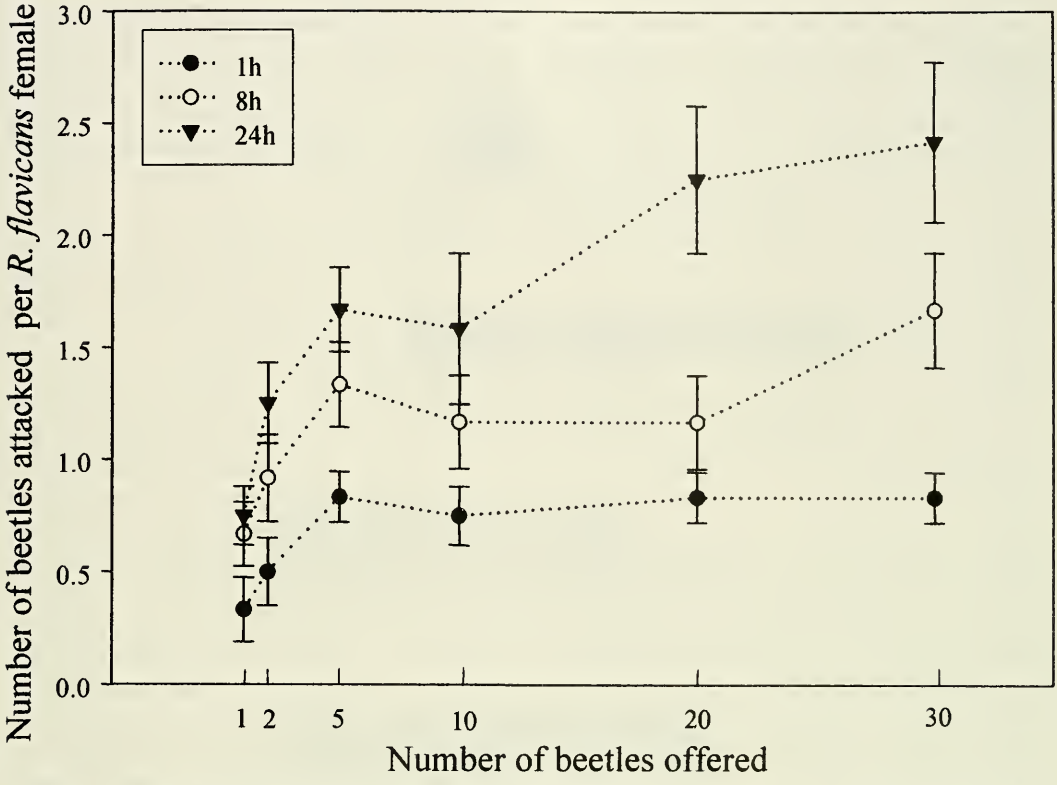

Fig. 1. Functional response of *Repipta flavicans* females in 1 h, 8 h and 24 h, to different densities of *Acalymma blomorum*, under laboratory conditions.

(Fig. 1), but eventually the rate of consumption remains constant regardless of increases in prey density. Predators consumed a maximum of 2.4 ± 0.86 (SE) prey in 24 h. A functional response curve of type II (Holling 1959) was observed.

Predation bioassays.—An immediate effect of cucurbitacins on the predators was not observed. Predators did not reject *A. blomorum* fed on CC diet. No mortality was observed in the control beetles. Females and males responded in a similar pattern to the prey species offered. However, significant preferences were obtained only for females, for the pair, *A. innubum* vs. *D. tibialis*, males did not prey on *D. tibialis* (Fig. 2).

1) Specificity. *Coloration:* When comparing predation on *A. blomorum* and *A. trivittatum* no significant difference was detected. Nevertheless, greater predation was observed on *A. blomorum* than on *C. atrofasciata* ($P = 0.005$). Finally, when *A. blomorum* and *D. balteata* were offered, predators preferred *D. balteata* ($P = 0.025$, Fig. 2). No significant difference in predatory behavior between female and male bugs was detected.

Body size: No preference for one of the larger species, *A. innubum* and *D. tibialis* was found (Fig. 2). Also, no difference in numbers of beetles preying upon the smaller or the larger species was found. Nevertheless, female *R. flavicans* preyed upon significantly greater number of beetles than did males ($P = 0.025$).

2) Effect of the diet of *A. blomorum* on predation by *R. flavicans*. No difference between treatments was found. Neither between predators collected in the field or raised in the laboratory, nor between females and males.

3) Effect of the diet of *A. blomorum* on the longevity of *R. flavicans*. The longevity recorded for female predators that ate *A. blomorum* fed on the SC diet was 66 ± 12. 8 d, whereas individuals which preyed on *A. blomorum* fed on the CC diet was 103 ± 12.3 d. The difference between treatments was significant ($P < 0.05$). Also, true bugs consumed more individuals of *A. blomorum* fed on the CC diet (83%) than of *A. blomorum* fed on the SC diet (75%, $P = 0.0044$).

Quantitative detection of cucurbitacins in *A. blomorum* and *R. flavicans*.—In chromatograms obtained from *A. blomorum* fed on the SC diet two spots were detected, one with Rf = 0.45 and a second with Rf = 0.14. In extracts from *A. blomorum* fed on the CC diet one spot with Rf value of 0.53 was obtained. From the extracts of *R. flavicans* fed with *A. blomorun* fed on the CC diet we observed one spots with Rf = 0.53. As standards we obtained for cucurbitacin B: Rf = 0.72, for cucurbitacin D: Rf = 0.41, and for cucurbitacin I: Rf = 0.55.

Discussion

In our study *Repipta flavicans* responded positively to prey density, but quickly became satiated, and was not specialized on *Acalymma* spp. Even though *R. flavicans* responded positively to prey density, its predatory behavior did not seem to be density dependent. An explication for this observation might be that the probability of success in the first attack is related to prey density. However, after the first prey is consumed, this predator seemed to be somewhat saturated and its subsequent prey captures were slower (Holling 1959). In addition, due to the long handling time necessary for capture and extraoral digestion, the average number of prey consumed was only 2.4 beetles in our 24 h assays, as well as in the 48 h longevity assays. Laboratory experiments by O'Neil (1997) showed, that the functional response of the *Podisus maculiventris* (Heteroptera: Pentatomidae) increased according to the density of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). This study, however, was done with the nearly immobile larvae of that species, and like in our study, handling time, rather than prey availability, limited the number of prey items that a predator can consume.

In the field, individuals of *R. flavicans* were observed to prey only on *A. blomorum,* although several Diabroticina species coexisted on the same hosts.

Under laboratory conditions, when comparing predation on *A. blomorum* and *A. trivittatum,* no significant difference was found between prey species, perhaps due to the similarity in coloration of their elytra and in body size. Nevertheless, *R. flavicans* females preferred *A. blomorum* over *C. atrofasciata,* and both, females and males preferred *D. balteata.* Therefore, the preference of *D. balteata* over *A. blomorum* observed in our bioassays was surprising. A factor that could have affected predation on these two species, was their activity level. According to observations in the laboratory, individuals of *D. balteata* were more active than *A. blomorum.* Once the bioassay was initiated, *D. balteata* beetles were constantly moving, which probably attracted the attention of the predator, since it walked behind the prey or waited until the beetle came within reach. By contrast, *A. blomorum* adults stayed motionless when they discovered the presence of the predatory bugs (Gámez-Virués et al. 2003).

When *A. innubum* and *D. tibialis* (both species are larger than *A. blomorum*) were offered, females preyed upon more beetles than did males. Also, males did not successfully attack *D. tibialis.* Female *R. flavicans* are larger than males (Gámez-Virués et al. 2003) and may have increased nutritional requirements due to egg production. The resulting costs might reflect the greater voracity of female bugs. Nevertheless, males tried to capture individuals of *D. tibialis,* but due to their size and perhaps also their weight, beetles managed to escape before the bug could introduce its stylets into

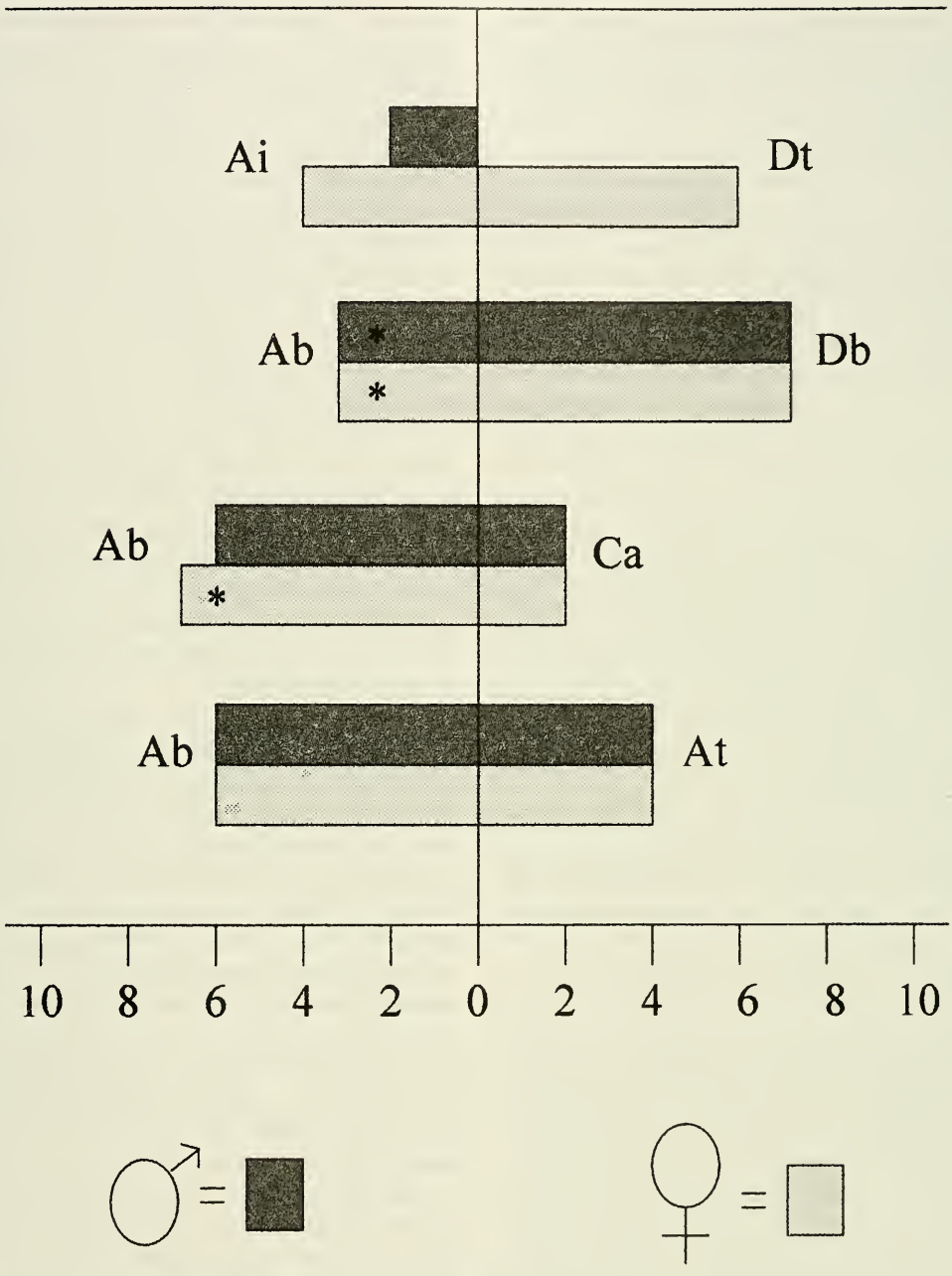

Fig. 2. Number beetles of *Acalymma blomorum* (Ab), *A. innubum* (Ai), *A. trivittatum* (At), *Cerotoma atrofasciata* (Ca), *Diabrotica balteata* (Db), and *D. tibialis* (Dt), predated by female and male *Repipta flavicans* in a choice bioassays. Significant difference ($P < 0.05$) between Diabroticina species are indicated with an asterisk (*).

the intersegmental membranes of the prey's abdomen. In addition, *D. tibialis* and *C. atrofasciata* beetles have shiny, wax-covered elytra, whereas the elytra of *A. blomorum* have distinct longitudinal ridges and a reticular pattern with punctures. Those morphological characteristics might allow *Repipta* bugs to capture them with greater

facility. Further experiments including beetles from other families are necessary to determine if this predator is truly a generalist.

Cucurbitacins are evidently aimed to repel vertebrate herbivores (DaCosta and Jones 1971). Some invertebrates like naive preying mantids rejected Diabroticina beetles that had fed during seven days on squash fruit with high concentrations of cucurbitacins (Ferguson and Metcalf 1985). Contrary to the chemical defense hypothesis, the presence of cucurbitacins in the beetles of our study did not affect the predatory behavior of *R. flavicans*. There are several studies that did not find negative effects of secondary plant compounds on species of the third trophic level (Barbercheck 1993, Down et al. 2003, Karimzadeh et al. 2004). On the other hand, Mitchell et al. (2004) observed that oviposition behavior of a parasitoid wasp was significantly slower due to secondary compounds in host eggs, but time for drilling, oviposition, and marking was unaffected as well as progeny emergence, longevity, or sex ratio.

Apparently *R. flavicans* benefited from the cucurbitacins in its prey, since its longevity was enhanced. It might be possible, that this predatory bug uses cucurbitacins sequestered from prey beetles for its own chemical defense. It is well known that harpactorine species emit volatile secretions with pungent scents as chemical defense (Ambrose 1999). In addition, numerous predacious Hemiptera have been observed to complement their diet with plant material, which can accelerate nymphal development, and increase longevity and fecundity (Ambrose 1999). Our observations agree with the data reported by Ambrose, since the longevity of *R. flavicans* was greater in individuals that ate *A. blomorum* fed on the CC diet. Furthermore, in the laboratory, we observed *R. flavicans* eating fruits of both cucurbit hosts. In a previous paper we described its laboratory biology and established a small scale rearing method (Gámez-Virués et al. 2003). If *R. flavicans* obtain a direct effect from cucurbitacins, we would expect to collect greater number of *R. flavicans* on *C. o. martinezii* (bitter cucurbit), but this does not agree with our field observations. We collected 0.54 individuals of *R. flavicans* per hour on *C. moschata* against 0.01 individuals on *C. o. martinezii* (unpubl. data). In addition, numbers of beetles collected on *C. moschata* were significantly greater than on *C. o. martinezii* (Gámez-Virués and Eben, 2005). Perhaps *R. flavicans* reacted more the abundance of its prey than to the non volatile secondary compounds that characterize many wild cucurbits. Moreover, perhaps beetles fed on CC diet adquire more nutrients than those fed on SC diet due to their compulsive behavior to cucurbitacins (Metcalf 1986). As consequence, *R. flavicans* reared on *A. blomorum* fed on CC diet obtained dietary benefits that improved its longevity. This hypothesis remains to be further investigated.

To our knowledge, no studies have been published that demonstrate a direct beneficial effect of non-volatile secondary compounds sequestered by herbivores on predators or parasitoids. Spiteller et al. (2000) found that beet armyworm oral secretions contain volicitin, a compound partly produced by the insect, partly metabolized from plant sequestered linolenic acid. Upon wounding, volicitin elicits volatile secretion in maize. Gentry and Dyer (2002) reported that caterpillars containing unpalatable chemicals were preferentially parasitezed. Those compounds then attract natural enemies of the herbivore.

According to our field observations, we know that *R. flavicans* are able to eat *A. blomorum* beetles found on wild, cucurbitacin-producing *Cucurbita* species as well as on non-bitter cultivars. Possibly as a result of the longterm coexistence of these species in their native habitat, this predator has adapted to the plant secondary compounds as well. This hypothesis might be further supported by our findings that these true bugs sequester cucurbitacins from their prey. More experiments are required to un-

derstand potential tritrophic level effects in the described interaction.

This study is the first that used a predatory hemipteran to test the chemical defense hypothesis in Diabroticina. Reduviidae have sucking mouth parts and use extraoral digestion. Perhaps when using invertebrate predators with a buccal apparatus different from true bugs, the rejection of *A. blomorum* with sequestered cucurbitacins would be evident, as has been reported in earlier studies for other Diabroticina species (Ferguson and Metcalf 1985, Tallamy et al. 1997, Tallamy et al. 1998). Ferguson and Metcalf (1985) reported that 100 % of *Acalymma vittatum* Fabricius collected in the field had extractable concentrations of cucurbitacins in their body. In Mexico, where numerous wild bitter cucucrbit species can be found, the percentage of *Acalymma* spp. that retain cucurbitacins under natural conditions is not known. We are currently gathering these data.

The compounds detected in the whole-body extracts of both beetle treatments and of *R. flavicans* chromatographically matched those of the cucurbitacins detected in the fruit extracts (Rf = 0.53, cucurbitacin I). It has been reported (Metcalf et al. 1982) that *C. o. martinezii* produces cucurbitacin E and I. The cucurbitacins with Rf value of 0.45 might have been another cucurbitacin: J or K glycoside reported for a closely related subspecies, *C. okeechobeensis* (Metcalf et al. 1982) or metabolites of the compound (Halaweish and Tallamy 1993), but standards were not available for confirmation. We are currently isolating the cucurbitacin profile of *C. o. martinezii* (unpubl. data). The chromatograms obtained from the whole body extracts of *A. blomorum* reared on the SC diet show that beetles maintain sequestered cucurbitacins longer than 28 d. Therefore, we were not able to compare between beetles with and without cucurbitacins, but rather made a comparison between beetles with different concentrations of sequestered compounds. In an earlier study, it was reported that *A. vittatum* excreted 67% of sequestered cucurbitacins after 48 h (Ferguson et al. 1985, Andersen et al.1988). No further experimental data have yet been published on the length of time cucurbitacins can be stored in the body of Diabroticina beetles.

Our results suggest that due to the slow predation and quick satiation, *R. flavicans* can probably not be used as biocontrol agent against Diabroticina in the field, but maybe in a greenhouse situation. Nevertheless, it might be important to conserve this predator for a multi-species approach to integrated pest management.

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